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Introduction

Ethanol has been made since ancient times by fermenting sugars. All the ethanol used for fuel and alcoholic drinks, and most industrial ethanol, is made by this process (Licht 2001). In 2002, world ethanol production was projected at 34 million m^3 (Licht 2002).

Fuel ethanol is also known as bioethanol, since it is produced from plant materials by biological processes. Fuel ethanol is the largest market by far, accounting for 60% of total ethanol production worldwide (Licht 2001). This share is likely to increase over the coming years as many countries set up fuel ethanol programmes. Industrial ethanol accounts for 20% of the market and beverages for about 15%; both these markets are growing comparatively slowly.

The world's largest ethanol producers are Brazil and the USA, which together account for more than 65% of global ethanol production; the figure for Europe is 13%. Fuel ethanol is produced in Brazil mainly from sugar cane and in the USA from corn, accounting for 11.9 and 7.6 million m^3 respectively in 2001 (Licht 2001). In the USA, ethanol has been used successfully in clean fuel programmes in Minnesota, Wisconsin, Oregon and the Chicago metropolitan area (Vaghn, 1999).

Because bioethanol is a renewable fuel it is commercial-available transport fuel that helps to reduce emmision of carbon dioxide (Vaghn 1999, Macedo 1998). Fossil fuels release carbon dioxide into the air when they are burned, but bioethanol is "CO_2-neutral" because the carbon dioxide released by burning is absorbed from the atmosphere by the next generation of crops used in the manufacture of bioethanol.

A recent report by Argonne National Laboratory concluded that, compared to gasoline, using ethanol from corn reduces the demand for fossil-fuel energy by 50–60% and cuts greenhouse gas production by 35–46%. For ethanol produced from cellulosic materials, these reductions are even greater (Vaghn, 1999).

Bioethanol as a fuel

Ethanol is a clear, colourless, flammable, oxygenated hydrocarbon with the chemical formula C_2H_5OH. Ethanol can be used as a transport fuel in at least four forms: anhydrous ethanol (100% ethanol), hydrous ethanol (95% ethanol and 5% water), anhydrous ethanol-gasoline blends (10–20% ethanol in gasoline) and as raw material for ethyl tert-butyl ether (ETBE) (Wyman and Hinman, 1990).

An anhydrous blend of 10% ethanol in gasoline (E10) is sold as "gasohol" in the USA and Canada. In Brazil, up to 90% of new cars have engines specially designed to run on hydrous ethanol. This avoids the expense of removing the remaining 5% of water, and also takes advantage of the fact that water increases the octane number and latent heat of evaporation of ethanol (Wyman and Hinman, 1990).

As a fuel, ethanol competes with gasoline (petrol), diesel and MTBE (methyl tert-butyl ether, added to gasoline at a concentration of 5–10% as an octane booster).

Compared to gasoline and diesel, ethanol is per litre more expensive and has a lower energy density, so more is needed to drive a given distance (Table 12). Compared to MTBE, however, ethanol is comparable regarding price per energy unit and has considerable environmental advantages.

Table 12. Heating values and prices (May 2003) of fossil fuels and ethanol.

Fuel	MJ/kg	MJ/l	Price* kr/l
Gasoline (regular 95)	42.7	31.4	2.15
Diesel	42.5	35.5	2.09
MTBE	35.2	26.7	3.10
Ethanol	27	21.4	2.47

*without taxes or transport

MTBE is added to gasoline as an octane booster, replacing the lead formerly used for this purpose, and to reduce emissions of smog-forming air pollutants. Because MTBE is made from fossil fuels, however, it is a net contributor to greenhouse gas emissions. MTBE is also a serious pollutant in groundwater because it is water-soluble, highly toxic and resists biodegradation. Ethanol biodegrades quickly in soil and water and is not toxic in small amounts (quantities).

It therefore seems clear that the biggest advantages will come from replacing MTBE with ethanol. However, ethanol has several advantages compared with gasoline and diesel as well as MTBE:

- positive net energy balance;
- less severe impact on the environment (both air and groundwater);
- less dangerous to health;
- reducing dependence on oil imports;
- helps maintain rural economies; and
- promotes biotechnology.

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Raw materials

Sugar is required to produce ethanol by fermentation. Plant materials (grain, stems and leaves) are composed mainly of sugars, so in principle almost any plants can serve as feedstock for ethanol manufacture.

In practice, the choice of raw material depends on what grows best under the prevailing conditions of climate, landscape and soil composition, as well as on the sugar content and ease of processing of the various plants available. The result is a wide variety of ethanol feedstocks, and hence production processes.

Most bioethanol is produced from sugar cane (Brazil), molasses and corn (USA), but other starchy materials such as barley, rye and wheat are also suitable. Bioethanol can also be produced from forest and agriculture residues such as wood chips and straw from corn, wheat, rye, oat, barley and rice. With a total sugar content of 60–70% (40% glucose as cellulose and 25% xylose as hemicellulose), wheat straw can produce around 230 kg of ethanol per tonne of dry material. Table 13 shows estimated ethanol yields from various feedstocks.

Ethanol production

The production of bioethanol requires two steps: fermentation and distillation. Practically all ethanol fermentation is still based on Baker's yeast (Saccharomyces cerevisiae), which requires simple (monomeric) sugars as the raw material. Conventional yeast fermentation produces 0.51 kg of ethanol from 1 kg of any the C_6 sugars glucose, mannose and sucrose (the last reaction in Figure 16).

Molasses is a by-product of the cane sugar and beet sugar industries. Compared with other feedstocks, molasses has the advantage that it contains around 50% of simple sugars that can be fermented directly to ethanol (Table 13) (Murtagh 1995).

However, not all feedstocks contain simple sugars. In grain, for example, glucose molecules are linked by α-1-4 bonds to create starch. Many plant materials contain lignocellulose, in which glucose molecules are linked by β-1-4-bonds. In both cases, hydrolysis (the addition of water, for instance by enzymes) is needed to break these bonds and produce simple C_6 sugars for fermentation (the first reaction in Figure 16).

Yeast and other microorganisms can also produce ethanol from simple C_5 sugars such as xylose, which is

Table 13. Raw materials, processing temperatures and enzymes for pre-hydrolysis, content of fermentable sugars and potential ethanol yields per 100 g dry weight.

Raw material	Temperature (°C) used for pretreatment/ enzymatic hydrolysis	Enzymes (type)	Hexoses (g/100g)	Pentoses (g/100g)	Ethanol potential (g/100g)
Sucrose and starch					
Molasses	None	None	50	0	32
Sugar cane	None	None	65	0	28
Corn	130–160/52	Amylases	76	0	32
Wheat	130–160/52	Amylases	72	0	31
Rice	130–160/52	Amylases	80	0	34
Rye	130–160/52	Amylases	70	0	30
Barley	130–160/52	Amylases	72	0	31
Potato	130–160/52	Amylases	56	0	24
Lignocellulose					
Bagasse	190–210/50	Cellulases	45	25	26
Corn stover	190–210/50	Cellulases	41	25	25
Wheat straw	190–210/50	Cellulases	37	25	23
Aspen	190–210/50	Cellulases	51	17	26
Willow	190–210/50	Cellulases	40	12	19
Spruce	190–210/50	Cellulases	61	5	25
Waste					
Municipal solid waste	190–210/50	Cellulases	42	18	23
Cellulose sludge	190–210/50	Cellulases	39	7	17

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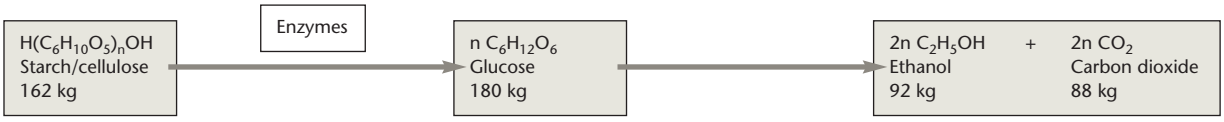

Figure 16. Hydrolysis of starch and cellulose followed by fermentation of glucose to ethanol using Bakers's yeast.

derived from hydrolysis of the polymer hemicellulose, itself a component of lignocellulose (McMillan, 1994b).
The hydrolysis step that precedes fermentation requires a combination of physical and chemical conditions that is normally specific to the type of material being processed (Wyman, 1994). In particular, starchy and lignocellulosic raw materials need different enzymes and hydrolysis regimes, so they are considered separately in the following sections.

Fermentation of starch to ethanol

Wet milling and dry milling

For fermentation processes based on starch, the raw material is usually some kind of grain. To release the starch, the grains must first be broken open. The two most widely used methods of doing this are wet milling and dry milling (Licht 2001).
In wet milling the grain is first steeped in a solution of water and sulphur dioxide for 24–48 hours at a temperature of around 52°C, and then passed through mills to loosen the germ and the hull fibres. In dry milling the grain is broken up into particles that are as small as possible, to facilitate subsequent penetration of water.

Hydrolysis and fermentation

Once milled, the starchy material must be "saccharified" to convert the starch into fermentable sugars. This is normally done with the help of enzymes known as amylases, whose job is to hydrolyse starch.
In its natural state, starch exists as compact crystalline granules that are resistant to enzymatic attack. To help the enzymes work better, heat is used to dissolve the starch molecules.
The milled grain is first made into a slurry in water. A small quantity of α-amylase is added to reduce the viscosity, and the slurry is then cooked at 130–160°C. Once the starch has gelatinised, the resulting "mash" is cooled to 80–90°C and the rest of the α-amylase is added, producing rapid liquefaction.
When the mixture has cooled to 32°C, a mixture of amyloglycosidase and yeast is added. Amyloglycosidase is an enzyme that performs the main hydrolysis step, after which the yeast converts the resulting simple sugars into alcohol.
This proces (Figure 16) of carrying out the enzymatic liberation of glucose and the fermentation in a single process step is known as SSF (simultaneous saccharification and fermentation).
Traditional fermentation, known as SHF (separate hydrolysis and fermentation), uses separate steps and different process conditions for the enzymatic pre-treatment and the fermentation. SSF gives higher yields because it minimises substrate (glucose) inhibition.

Fermentation of lignocellulose to ethanol

Lignocellulosic materials such as straw and wood, which are often available as wastes, are much cheaper than grain. Converting them to ethanol, however, requires complex and costly processes. For lignocellulosic materials to become economic as ethanol feedstocks requires the development of new technologies.
Lignocellulosic materials contain two types of polysaccharides, cellulose and hemicellulose, bound together by a third component, lignin. From the point of view of ethanol fermentation, they are hard to work with for two reasons. First, the lignin protects the cellulose and hemicellulose from attack by enzymes. Second, when enzymes do manage to reach the cellulose and hemicellulose they are hindered by the crystalline structure of these molecules.

Pre-treatment

The first step in processing lignocellulosic materials is a pre-treatment step in which some of the hemicellulose dissolves in water, either as monomeric sugars or as oligomers and polymers. The temperature range is normally 150–200°C. The main processes are:

- steam explosion;
- treatment with ethanol/water mixtures (the Organosolv process); or
- high-temperature/high-pressure treatment with acid alkalis, oxygen or both.

This is followed by treatment with enzymes known as cellulases and hemicellulases, which hydrolyse cellulose and hemicellulose respectively. The effectiveness of the enzymes depends on their origin (Thygesen et al. 2003), the nature of the previous treatment step(s) and the properties of the feedstock, notably the degree of cellulose crystallinity and the amount and type of lignin. Pretreatment using alkali and oxygen (wet oxidation) effectively removes lignin without producing toxic compounds and seems to give the best performance at the enzyme treatment stage when treating annual crops like wheat straw (Bjerre et al., 1996; Klinke et al., 2002, 2003).

Hydrolysis and fermentation

Following pre-treatment, the next step is to use enzymes to hydrolyse the cellulose fraction and release glucose. This step takes place at 50°C, with the enzymes added as

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Xylose → 30% Ethanol + 8% Acetate + 2% Lactate + 40% CO + 20% Hydrogen

Figure 17. Conversion of xylose to ethanol by the thermophilic bacterium Thermoanaerobacter mathranii.

a mixture of cellulase and β-glucosidase. The actual fermentation is a two-stage process. In the first stage, glucose is fermented at 32°C with traditional Baker's yeast (Figure 16). As in starch fermentation, enzymatic hydrolysis and fermentation can be carried out simultaneously (the SSF process).

The second fermentation step converts pentoses – mainly xylose – into ethanol. This is done using special genetically-modified microorganisms or selected natural strains (Zaldivar et al., 2001) (McMillan, 1994a). The anaerobic bacterium Thermoanaerobacter mathranii (figure 17), discovered in a hot spring in Iceland, can convert xylose to ethanol at 70°C (Larsen et al., 1997). A genetically-modified Escherichia coli has also been developed to convert all the sugars present in lignocellulosic hydrolysates to ethanol (Beall et al. 1991).

Co-production of bioethanol and biogas

Since 1994 the Technical University of Denmark and Risø National Laboratory have been co-operating on a new technology for producing both bioethanol and biogas (Figure 18) (Ahring and Thomsen, 2000). Such a process would eliminate the disadvantages of conventional, separate, bioethanol and biogas plants.

Conventional biogas plants use only 50% of their feedstock. The remainder consists mainly of lignocellulosic materials, which make up a large proportion of animal manure. These pass almost unconverted through the biogas plant.

Bioethanol plants, on the other hand, are designed to work with starch or celluloses. Lignins and other components which cannot be turned into fermentable saccharides are treated as effluent, which itself requires a further cleanup process, or at best burned as low-quality boiler fuel.

Co-production of bioethanol and biogas would allow all the components of both plant biomass and animal manure to be used. The wastewater from the ethanol plant, containing lignin and its oxidation products, as well as by-products of fermentation, acts as a secondary feedstock for the biogas reactor, resulting in a reduced cost price for ethanol of approximately 35% due to biogas production.

Perspectives

In summary, bioethanol is a renewable fuel that can reduce dependence on foreign energy, stimulate the rural economy, cut emissions of greenhouse gases and reduce contamination of waterways and groundwater following accidental spills.

In USA, the market on bioethanol is driven by the political out-phasing of MTBE. In Europe, a new directive

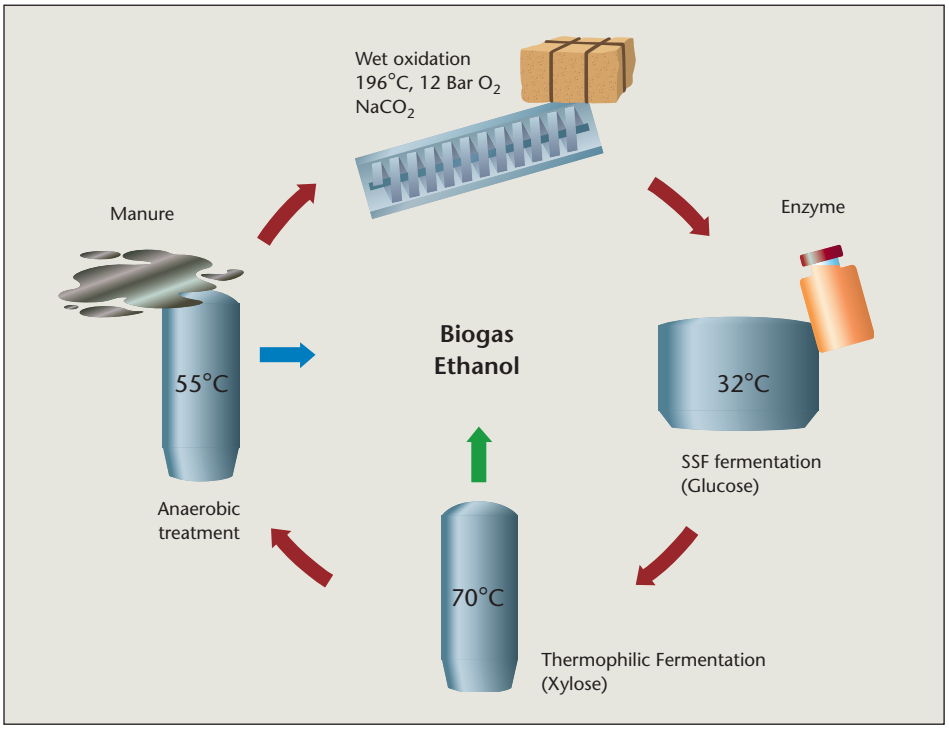

Figure 18. Danish Bioethanol Concept: straw and manure are used to produce bioethanol and biogas in an integrated plant that recycles process water.

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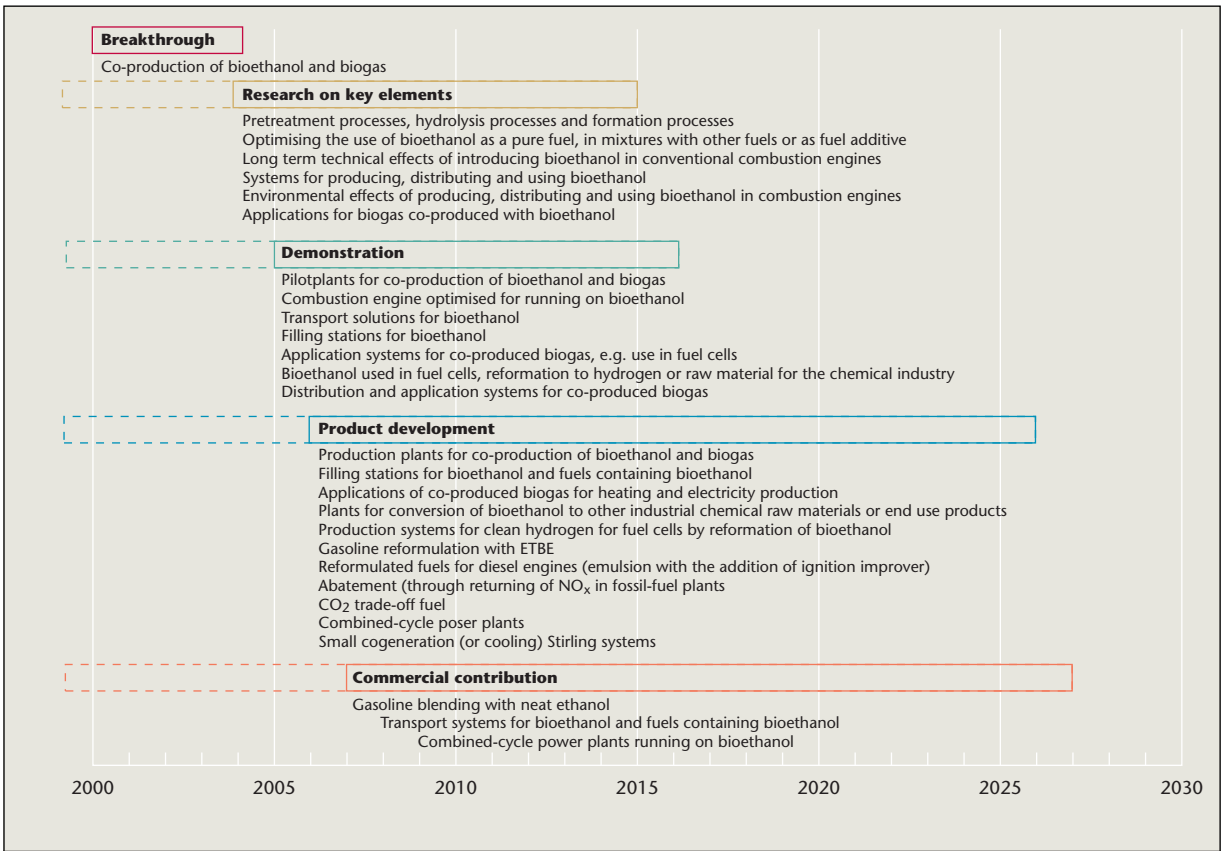
concerning sustainability of fuel and CO_2 reduction in the transport sector suggests that, in 2005, 2% of all gasoline and diesel is substituted by biofuels e.g. bioethanol, and in 2010, 5.75% will be substituted.
MTBE can be replaced by ethanol. A total substituting of MTBE in USA and in Europe according to the suggestions of the directives creates a new ethanol market on respectively 53 and 12 billion litres of ethanol per year (Table 14). This need will be difficult to meet by the conventional ethanol production methods without increasing the prices on corn and wheat. Bioethanol based on fermentation of biomass (in form of waste and energy crops) is a solution to this problem. However more research is still needed especially to reduce the cost or efficiency of commercial enzymes or, as another option, more efforts should be made to produce on-site enzymes as a part of the ethanol production. It has been shown that enzymes produced on the biomass to be used as raw material for ethanol fermentation are more efficient than commercial enzymes grown on artificial substrates (Thygesen et al 2003).

Table 14. Estimation of bioethanol production for transport and number of plants in 1005 and 2010 (numbers from IEA/DOE).

EU and USA bioethanol forecast	2005 EU/USA	2010 EU/USA
Gasoline consumption per year (bill. litres, IEA/DOE numbers)	145/545	142/619
Target case (2% in 2005 and 5,75% in 2010)		
Bioethanol required volume basis (bill. litres/year current trend)	4.3/16	12/53
Number of conventional ethanol plants required (at 250 bill. Litres/year using corn or wheat)	4/65	11/213
Percentage of required crops allocated for ethanol (wheat, corn, barley, sugar beets)	7/15	20/49

Figure 19. Bioethanol technologies and their time scale from breakthrough to commercial contribution.



■ Dotted areas indicates, that bioethanol produced by old-fashioned techniques is used for limited purposes in countries like Brazil and USA.